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Variation in Some Blood Parameters of Geese Subjected to Feather Gathering

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Abstract: Two experiments were conducted on 50 (25♂, 25♀) geese of Babat Hungarian Upgraded breed during their post-breeding moulting period to measure stress response to feather gathering. They were assigned to five treatment groups in both experiments: (1) control, (2) gathered, (3) given anti-stress supplement before gathered, (4) sham gathered and (5) given anti-stress supplement before sham gathered. In Experiment 1 blood samples were taken one hour before and one hour after the procedures and plasma levels of thyroxin (T_4) and triiodothyronine (T_3) were measured by radioimmunoassay. The concentrations of both T_4 and T_3 decreased post-exposure indicating a mild to moderate distress. In Experiment 2 blood samples were taken 24 h and 7 days post-procedures for determining total white blood cell counts and the heterophil granulocyte to lymphocyte (H/L) ratios. Feather gathering caused a mild stress that affected circulating white blood cells. However, the H/L method on its own is not a reliable stress indicator of the feather gathering procedure. The anti-stress supplement seemed to have little effect on the thyroid hormones levels or the haematological variables.

Key words: Goose, heterophil to lymphocyte ratio, thyroxin, triiodothyronine

INTRODUCTION

Gathering feathers from live geese is a traditional practice in Hungary regulated by the MARD (178/2009). (XII.29.), yet animal rights activists campaigned lately against this practice in the media for causing to geese pain and distress (Kozak *et al*., 2010).

Feathers are gathered from geese during their natural moulting when they shed and re-grow feathers. Young geese at 10-11 weeks of age and breeder geese after the egg-laying season undergo one complete moult followed by three partial moults every 6-7 weeks (Kozak, 2011). Both feather growth and the shedding of feathers (moult) are under endocrine control. Thyroxin (T_4) released by the thyroid gland stimulates the growth of young feathers and the actual shedding of feathers may result from a fall in the sex steroids at the end of the breeding season, when thyroxin secretion is above normal (Campbell and Lack, 2013).

Although, geese are more stress-prone during moulting than at other times the feathers can be gathered painless, without causing lesions to the skin. The new generation of feathers pushes the old out of the living tissue, so that the old feathers contain no living tissue and are not linked to the circulation or innervations. Any intimate connection between the base of the feather and the feather follicle is interrupted. The goose skin is relatively insensitive but the timing of gathering is crucial since feathers need to have "maturity" (Rauch *et al*., 1993).

The hypothalamic-pituitary-adrenal (HPA) axis plays a pivotal role in triggering the stress response. Physiological indicators of acute stress include the rapid secretion of catecholamines (adrenalin, noradrenalin) from the adrenal medulla and glucocorticoids from the adrenal cortex (Sapolsky, 1992). In birds, plasma levels of catecholamines can increase within seconds of exposure to stress and glucocorticoids rise within minutes (Le Maho *et al*., 1992). Elevation of adrenalin leads to increased vasoconstriction, heart rate, blood pressure and blood glucose (Freeman, 1985; Goldstein, 1987; Wittmann, 1994). Elevation of corticosterone leads to a series of events that can enhance short-term survival, including redirected behaviour and mobilization of energy reserves (Wingfield *et al*., 1998).

Similarly to the HPA axis the hypothalamic-pituitary-thyroid (HPT) axis is also stress-responsive. Its principal components are the hypothalamic thyrotropin-releasing hormone (TRH), pituitary thyroid-stimulating hormone (TSH) and the thyroid hormones: thyroxin and triiodothyronine (T_3). Thyroid hormones influence major processes such as growth, differentiation and metabolism (Norris and Carr, 2013). Stress regulation of the HPT axis has received little attention. Thyroid function is usually down-regulated during stressful conditions; triiodothyronine and thyroxin levels decrease with stress. Namely, stress inhibits TSH secretion through the action of glucocorticoids (end product of HPA axis) on the central nervous system (Helmerich *et al*., 2005).

Another indicator of stress is the ratio of heterophil granulocytes to lymphocytes (H/L) in the blood (Gross and Siegel, 1983; Maxwell, 1993). Stressors (e.g., food or water deprivation, temperature extremes, constant

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light, or exposure to novel social situations) elevate the number of heterophil granulocytes and depress the number of lymphocytes (Gross and Siegel, 1986; Gross, 1989; McFarlane and Curtis, 1989). Leukocyte numbers change more slowly (30 min to 20 h) in response to stress than does corticosterone (Dein, 1986; Maxwell, 1993; Cunnick *et al*., 1994). Further, these changes are less variable and longer lasting than the corticosterone response and multiple stressors usually have an additive effect (McFarlane and Curtis, 1989; McKee and Harrison, 1995). The mechanisms mediating these cellular changes are poorly defined in birds (Dohms and Metz, 1991), but may include changes in adrenal corticotrophin hormone and corticosterone (Gross and Siegel, 1983; Gross, 1989) and/or altered production of and responsiveness to cytokines (Cunnick *et al*., 1994). Previous examination of plasma glucose and corticosterone levels revealed that gathering feathers caused not more distress to geese than the handling or catching (Janan *et al*., 2001; Toth *et al*., 2012). This has prompted us to examine the thyroid hormones' levels and the heterophil granulocyte to lymphocyte ratio as a stress/welfare indicator for geese subjected to feather gathering with or without given an anti-stress supplement.

MATERIALS AND METHODS

Two experiments were conducted on 50 (25♂, 25♀) geese of Babat White Hungarian Upgraded breed during their post-breeding moulting period in early July. They were housed in pens on deep litter system with yard access and fed *ad libitum* a maintenance ration appropriate to the EU regulation (Council of Europe, 1999).

The geese in both experiments were assigned to one of five groups of 10 (5♂, 5♀) according to the following treatment protocol:

Group 1: Geese moulted naturally (control)
Group 2: Geese were subjected to feather gathering
Group 3: Geese given the anti-stress supplement from 5 days before gathering feathers
Group 4: Geese were subjected to sham feather gathering
Group 5: Geese given the anti-stress supplement from 5 days before sham gathering

The feather gathering procedure was performed on geese between 9:00 and 10:00 am. The geese were positioned dorsally with the head downwards, while feathers and down were removed from the lower belly, the flanks and the areas not covered by the wings. Subsequently, geese were turned on their ventral side and feathers were removed from the back (Szentirmay, 1968). The duration of this procedure including catching was about 10 min per goose. Sham feather gathering was done similarly without removing any feathers and it lasted for 4-5 min. The anti-stress supplement containing all essential amino acids and vitamins was given in the drinking water at a dose of 1 ml/goose/day.

In Experiment 1, the effect of feather gathering on the thyroid hormones' level was examined. Blood was taken from the wing vein into heparinized tubes one hour before and one hour after the said procedure during the forenoon to eliminate any diurnal variation in thyroid hormones (Newcomer, 1974); thyroxin (T_4) is highest during the night, triiodothyronine (T_3) is at its highest during the day (Klandorf *et al*., 1978). Plasma samples were obtained by centrifugation and stored at -20°C until analyzed. Plasma levels of thyroxin (T_4) and triiodothyronine (T_3) were determined by radioimmunoassay according Pethes *et al*. (1978).

In Experiment 2, the effect of feather gathering on total and differential white blood cell count was examined. Blood was taken from the wing vein into native collection tubes 24 h and 7 days post-procedures taking into consideration that exposure to a short-term physical stressor resulted fluctuations in heterophil to lymphocyte (H/L) ratio within 18 h; the response peaked about at 24 h and returned to normal at about 30 h in chickens (Gross, 1990). Total white blood cell (WBC) count was determined with a Bürker chamber using a WBC diluting fluid described by Horvath (1979). Differential count (relative percentage of each type of white blood cells) was appraised from 100 cells of May-Grunwald and Giemsa stained blood smears prefixed in methanol. The H/L ratio was calculated by dividing the sum of heterophil types by the sum of lymphocyte types (Gross and Siegel, 1983).

The blood variables were statistically processed using Microsoft Office Excel 2007 employing analysis of Variance (ANOVA) and the Student's t-test to reveal the significance of between or within-group differences in the means (Svab, 1981).

RESULTS

There were no significant between-sex differences in the means of the blood variables, therefore the group means are presented to give an overall view.

Mean plasma levels of thyroid hormones are shown by group and sampling time in Table 1. Mean plasma T_4 levels showed no significant between-group difference one hour before the feather gathering or sham gathering procedure ($p>0.1$). The values decreased in all five groups one hour post-procedures that was significant in group 3 and 5 ($p<0.10$, $p<0.05$; respectively). The decrease was lowest in group 1 (4%) comparable in groups 4 and 2 (7.4 and 7.5%, respectively) and highest in groups 3 and 5 (10.3 and 12.5%, respectively).

Mean plasma T_3 levels varied non-significantly across all the five groups one hour before the procedures. The

Table 1: Plasma levels of thyroid hormones by goose group and sampling time (Mean±SD)

	Group				
Time of sampling	1	2	3	4	5
			T_4 (mmol/l)		
Before	38.0±5.9	36.1±6.2	38.7±4.0[a]	39.2±5.0	40.0±4.0[b]
After	36.4±3.6	33.4±5.6	34.7±5.1[a]	36.3±5.0	35.0±3.0[b]
			T_3 (mmol/l)		
Before	2.7±0.6	2.4±0.7	2.2±0.5[b]	2.7±0.7[b]	2.2±0.5[b]
After	2.2±0.5	1.9±0.6	1.6±0.5[b]	2.0±0.6[b]	1.6±0.5[b]

Group 1: control, 2: feather gathering, 3: supplement+gathering, 4: sham gathering, 5: supplement+sham gathering
The differences in the same columns were significant at a = p<0.10, b = p<0.05 level

Table 2: Total white blood cell count, percentage of heterophil granulocytes and lymphocytes and H/L ratio by goose group and sampling time (Mean±SD)

	Group				
Time of sampling	1	2	3	4	5
			Total WBC count (G/l)		
24 h	15.8±1.5	15.6±1.0	15.4±1.0	15.3±1.0	15.2±1.0
7 days	15.9±10	15.7±1.0	16.7±1.0	15.6±1.0	14.8±1.0
			Heterophil granulocyte (%)		
24 h	37.0±3.0	37.0±3.0	37.0±3.0	39.0±3.0	38.0±3.5
7 days	38.0±2.5	36.0±2.0	36.5±4.0	37.0±2.0	36.0±2.0
			Lymphocyte (%)		
24 h	58 5±4.0	58.0±3.0	59.0±3.0	56.0±3.0	58.0±4.0
7 days	56.5±3.0[a,b]	60.0±3.0[b]	59.0±3.0[a]	58.5±3.0	60.0±2.0[b]
			H/L ratio		
24 h	0.64	0.64	0.63	0.70	0.66
7 days	0.67	0.60	0.62	0.63	0.60

Group 1: control, 2: feather gathering, 3: supplement+gathering, 4: sham gathering, 5: supplement+sham gathering
The differences in the same row were significant at a = p<0.10, b = p<0.05

values decreased in all five groups one hour post-procedures that was significant in groups 2, 4 and 5 (p<0.05). The decrease was lower in groups 1 and 2 (18.5 and 20.8%, respectively), while it was higher in groups 4, 3 and 5 (25.9 and 27.3, 27.3%, respectively).

Mean total WBC counts, percentage of heterophil granulocytes and lymphocytes and the H/L ratios are shown by group and sampling time in Table 2. Mean total WBC counts showed non-significant variation by either groups or sampling time (p>0.10). Mean percentage of heterophil granulocytes was identical in groups 1, 2 and 3, respectively and slightly higher in groups 4 and 5 at the 24 h post-procedure sampling. It was higher in group 1 compared to the other groups 7 days later. Mean percentage of lymphocytes varied non-significantly at the 24 h post-procedure sampling. However, 7 days later it was significantly lower in group 1 (p<0.10) than in the other groups, except group 4. The H/L ratio ranged between 0.63-0.70 at the first and 0.60-0.67 at the second sampling time.

DISCUSSION

The avian hypothalamus secretes TRH during stress and periods of decreased circulating hormones (Engelking, 2012). Thyroid hormones are also involved in stress regulation (Klandorf *et al*., 1978).

Previous studies suggested that stress causes a decrease in thyroid hormones, but the results are inconclusive. Immobilization has been shown to both increase and decrease thyroid hormone levels (Turakulov *et al*., 1994; Langer *et al*., 1983). In force-fed geese plasma level of both T_4 and T_3 was lower, than in birds fed naturally due to a possible metabolic adaptation to energy consumption of the body (Janan unpublished). The stress-induced decrease in thyroid hormones appears to be mediated, in part, by changes in hypothalamic drive to the axis (Helmerich *et al*., 2005). In this study, the consistent decreases in plasma T_4 and T_3 levels across all the five goose groups suggested a stress response. The small decrease in the control group possibly indicated a mild distress due to blood sampling, while the higher degree of decreases in the other groups may have caused a moderate distress associated with the uneasiness of feather gathering or sham gathering beyond blood sampling.

The application of heterophil to lymphocyte (H/L) ratios to assess stress has its origin in the 1980s. However, the inherent variation in leukocyte cell counts among individuals limits the utility of this method. Research with birds also demonstrated a relatively slow leukocyte response time that makes difficult obtaining baseline samples convenient (Davis *et al*., 2008).

The experimental treatments used in the Gross (year=1989) study altered the H/L ratios without having much effect on the white blood cell count. In our experiment total WBC counts, percentage of heterophil

granulocytes and lymphocytes varied relatively little 24 h pre- and 7 day post-procedures. A low H/L accompanied by a high total leukocyte count, a frank indicator of stress (Cotter, 2015), could not be detected in our sample.

In birds, the H/L ratio is normally less than 1. Gross and Siegel (1993) suggested that reference values for the H/L ratio is about 0.2, 0.5 and 0.8 are characteristic of low, optimal and high degree of stress in chickens. Based on this affirmation, geese in our experiment showed H/L values above the optimal (0.60) and below the high degree of stress (0.70). However, these figures corresponded to the physiological H/L ratios reported for domestic geese of 0.78 (Bardos, 2000) and for adult Babat Hungarian Upgraded geese of 0.55 (Nikodemusz *et al*., 1991). Thus the application of the H/L ratio of chickens (Gross and Siegel, 1983) may not be appropriate for domestic geese.

The results obtained here indicate the inadequacies of the H/L method, used alone, as a reliable stress indicator of gathering feathers from geese. This procedure resulted in a milder stress response that affected the circulating white blood cells.

The anti-stress supplement seemed to have little effect on the thyroid hormones levels or the haematological variables. The study of Carew *et al*. (1998) also showed that amino acid excesses had little effect on plasma levels of thyroid hormones in broiler chicks. A similar anti-stress supplement also showed no effect on plasma corticosterone levels in growing geese subjected to the feather gathering or shame gathering procedure (Toth *et al*., 2012).

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